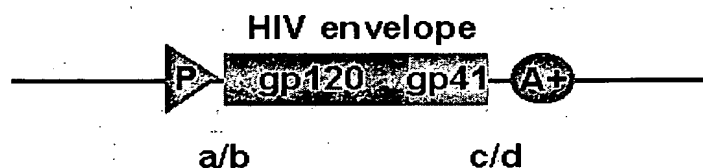


METHODS

Envelope Expression Vector: pHIVenv



HIV-1 Expression Vector: pHIVlucΔU3

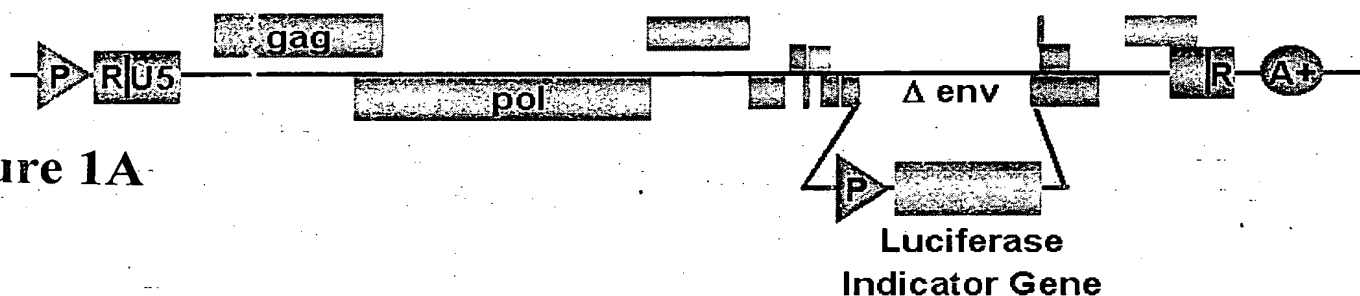


Figure 1A

PhenoSense HIV Entry Cell Assay

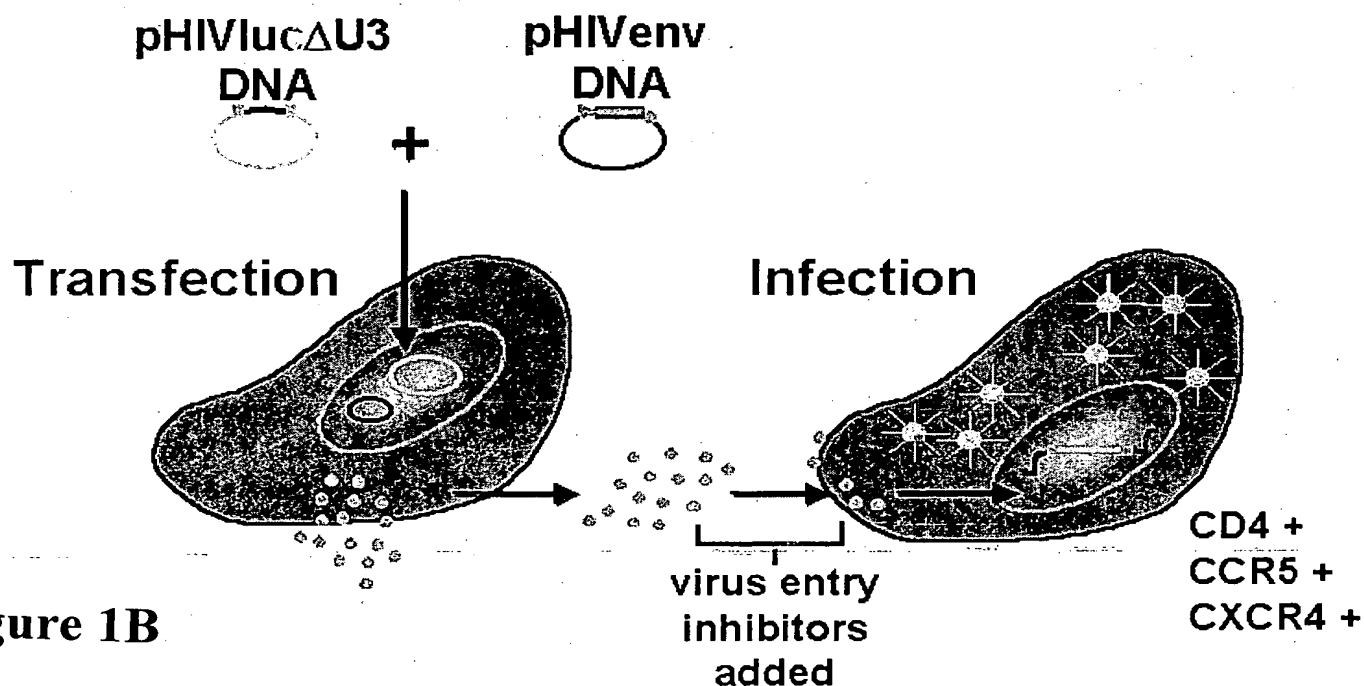
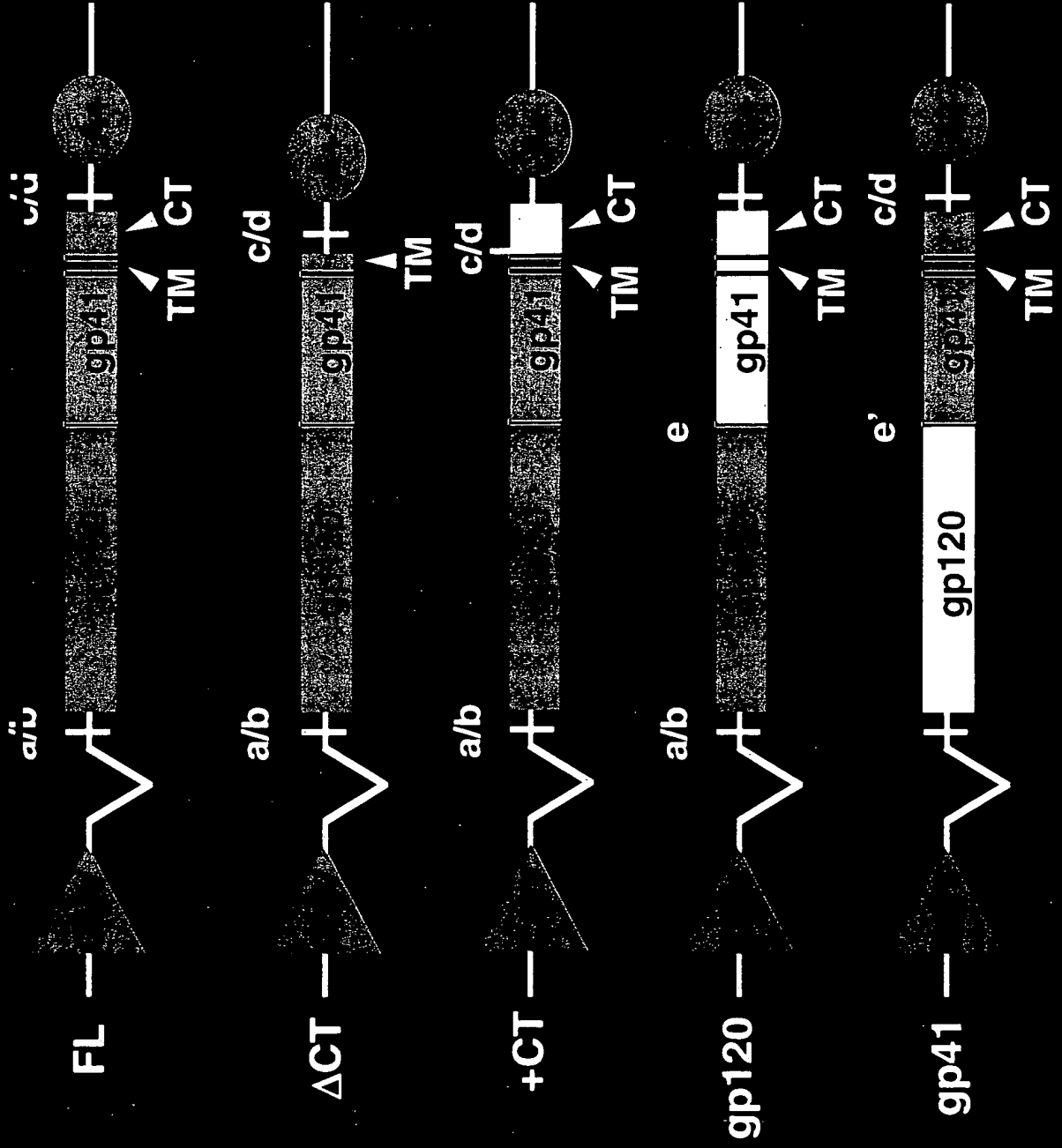
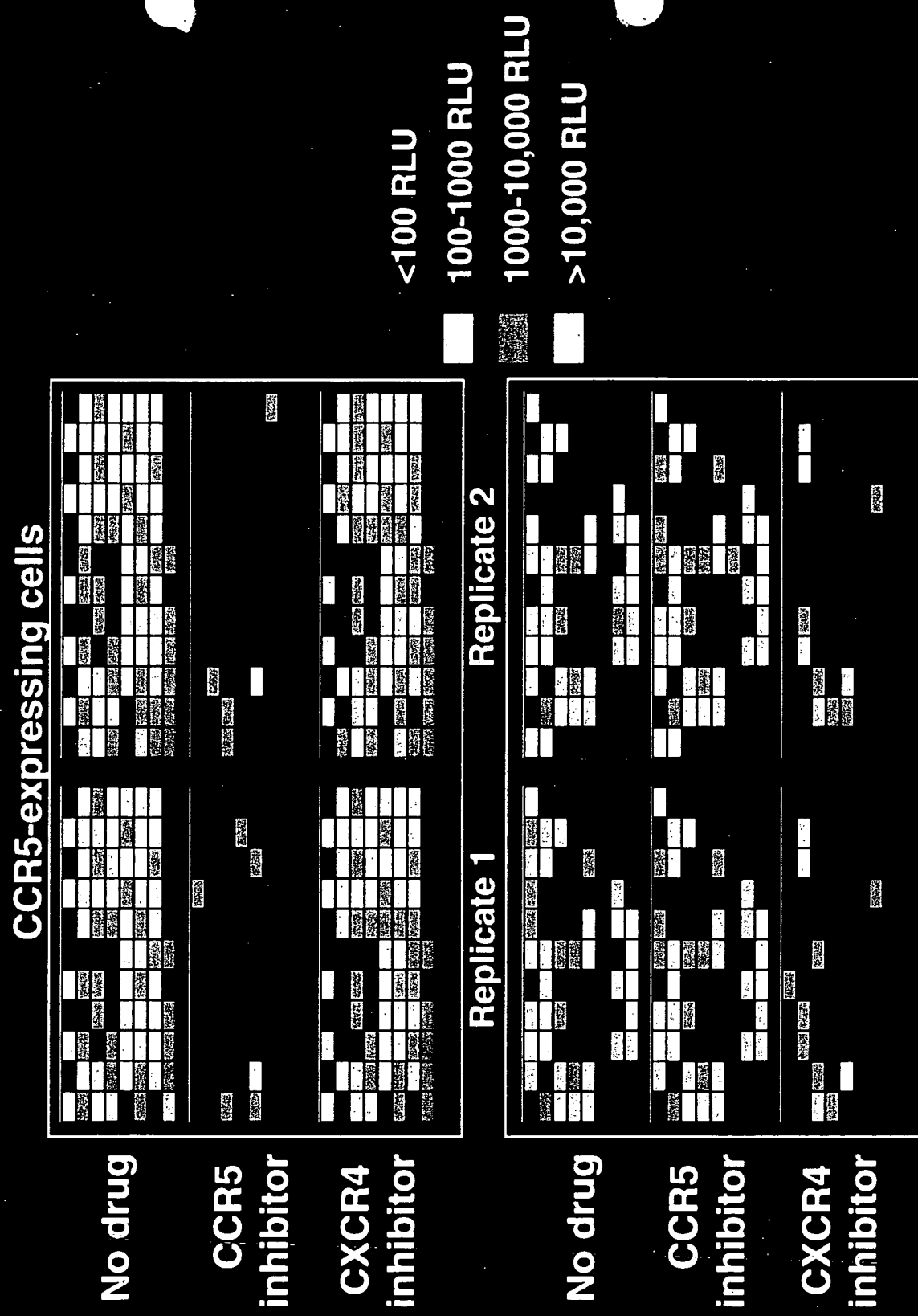


Figure 1B

HIV Envelope Expression Strategies



Co-Receptor Tropism Screen



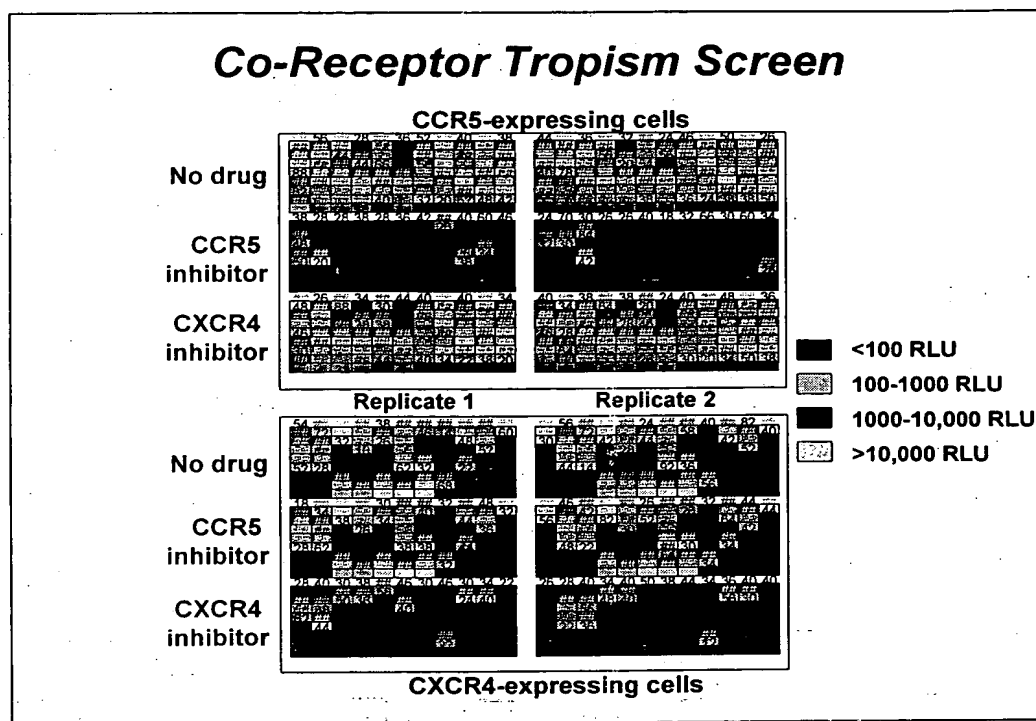


Figure 3A. Co-receptor Tropism Screening Assay

In this embodiment, the assay is performed using two cell lines. One cell line expresses CD4 and CCR5 (top six panels). The other cell line expresses CD4 and CXCR4 (bottom six panels). The assay is performed by infecting cells with a large number of recombinant virus stocks derived from cells transfected with pHIVenv and pHIVlucΔU3 vectors. The example shown represents the analysis of 96 viruses formatted in a 96 well plate. Infections are performed in the absence of drug (no drug), or in the presence of a drug that preferentially inhibits either R5 tropic (CCR inhibitor) or X4 tropic (CXCR4 inhibitor) viruses. Co-receptor tropism is assessed by comparing the amount of luciferase activity produced in each cell type, both in the presence and absence of drug (see Figure 3B for interpretation of assay results).

Co-Receptor Tropism Assay Interpretation

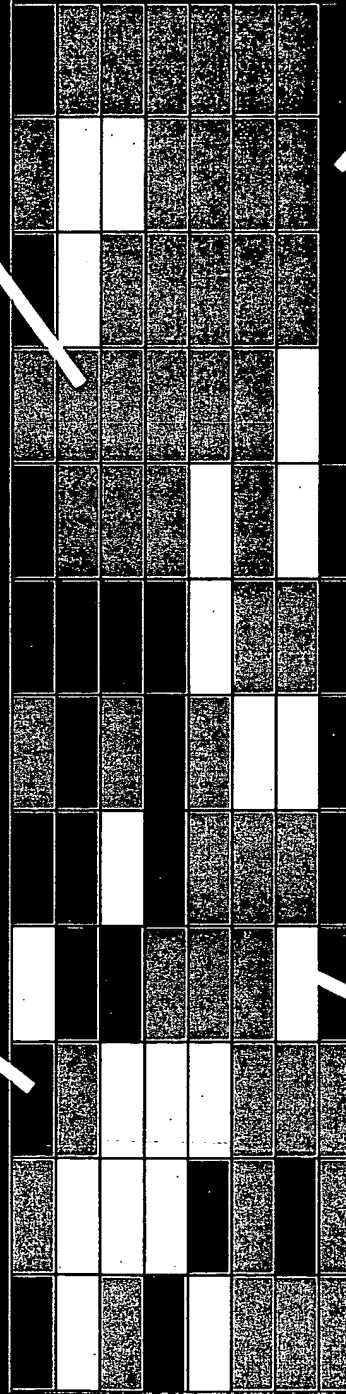
3

R5 cells	X4 cells	R5:X4
no drug		
R5 inhibitor		
X4 inhibitor		
%inhib by R5 inhibitor		
%inhib by X4 inhibitor		

21

R5	X4	R5:X4
17,381	72	936
99		
12,935		
100		
37		

no drug
R5 inhibitor
X4 inhibitor
%inhib by R5 inhibitor
%inhib by X4 inhibitor



CCR5
DUAL or MIXED
NON-VIABLE
active RLU limit: 100
tropism ratio limit: 5

76

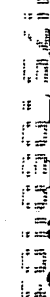
R5	X4	R5:X4
14,982	12,020	1
111	10,839	
8,580	3,384	
99	10	
43	72	

no drug
R5 inhibitor
X4 inhibitor
%inhib by R5 inhibitor
%inhib by X4 inhibitor

95

R5	X4	R5:X4
43	42	

no drug
R5 inhibitor
X4 inhibitor
%inhib by R5 inhibitor
%inhib by X4 inhibitor

[illegible]

100-443886-1

100-443887-100

[illegible]

100-443887-100

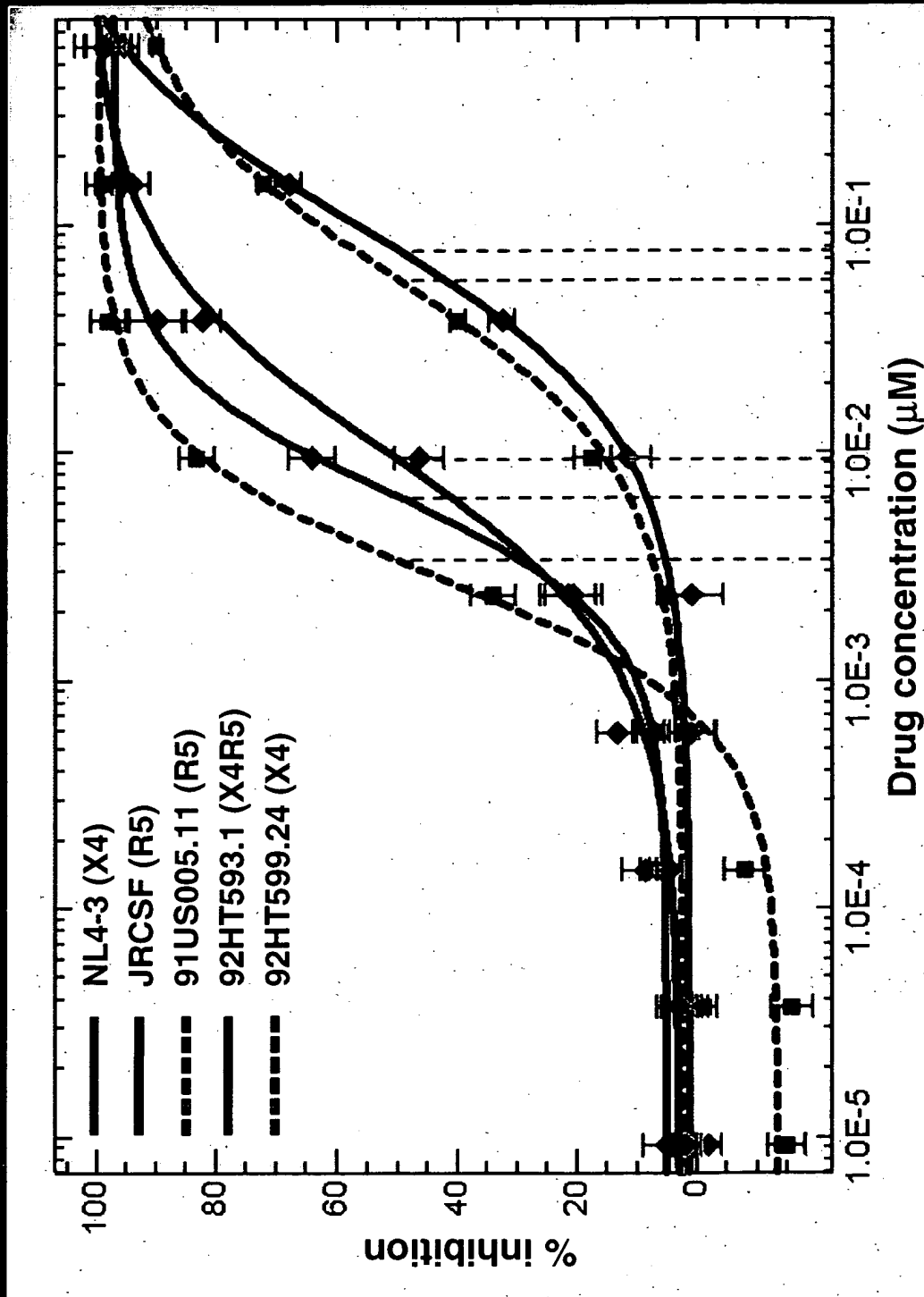
SECRET

[illegible]

100-443886-1

Figure 4A

Entry Inhibitor Susceptibility: Fusion Inhibitor



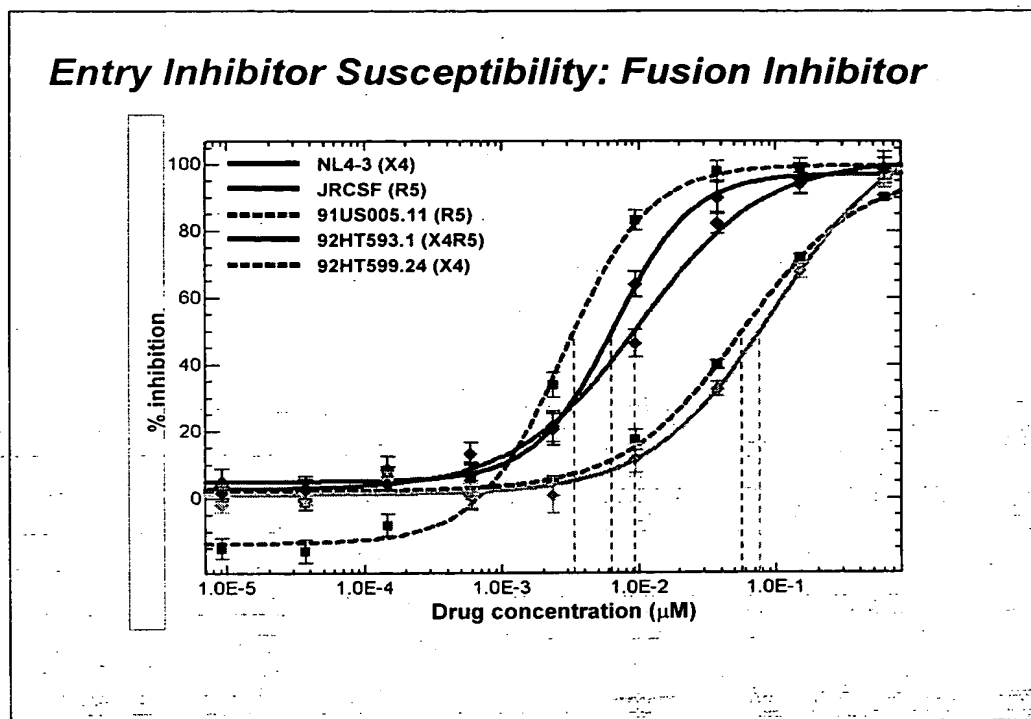


Figure 4A.

Measuring Entry Inhibitor Susceptibility: Fusion Inhibitor

In this embodiment, susceptibility to the fusion inhibitor T-20 is demonstrated. Cells expressing CD4, CCR5 and CXCR4 were infected in the absence of T-20 and over a wide range of T-20 concentrations (x-axis log10 scale). The percent inhibition of viral replication (y-axis) was determined by comparing the amount of luciferase produced in infected cells in the presence of T-20 to the amount of luciferase produced in the absence of T-20. R5 tropic, X4 tropic and dual tropic viruses were tested. Drug susceptibility is quantified by determining the concentration of T-20 required to inhibit 50% of viral replication (IC50, shown as vertical dashed lines). Viruses with lower IC50 values are more susceptible to T-20 than viruses with higher IC50 values.

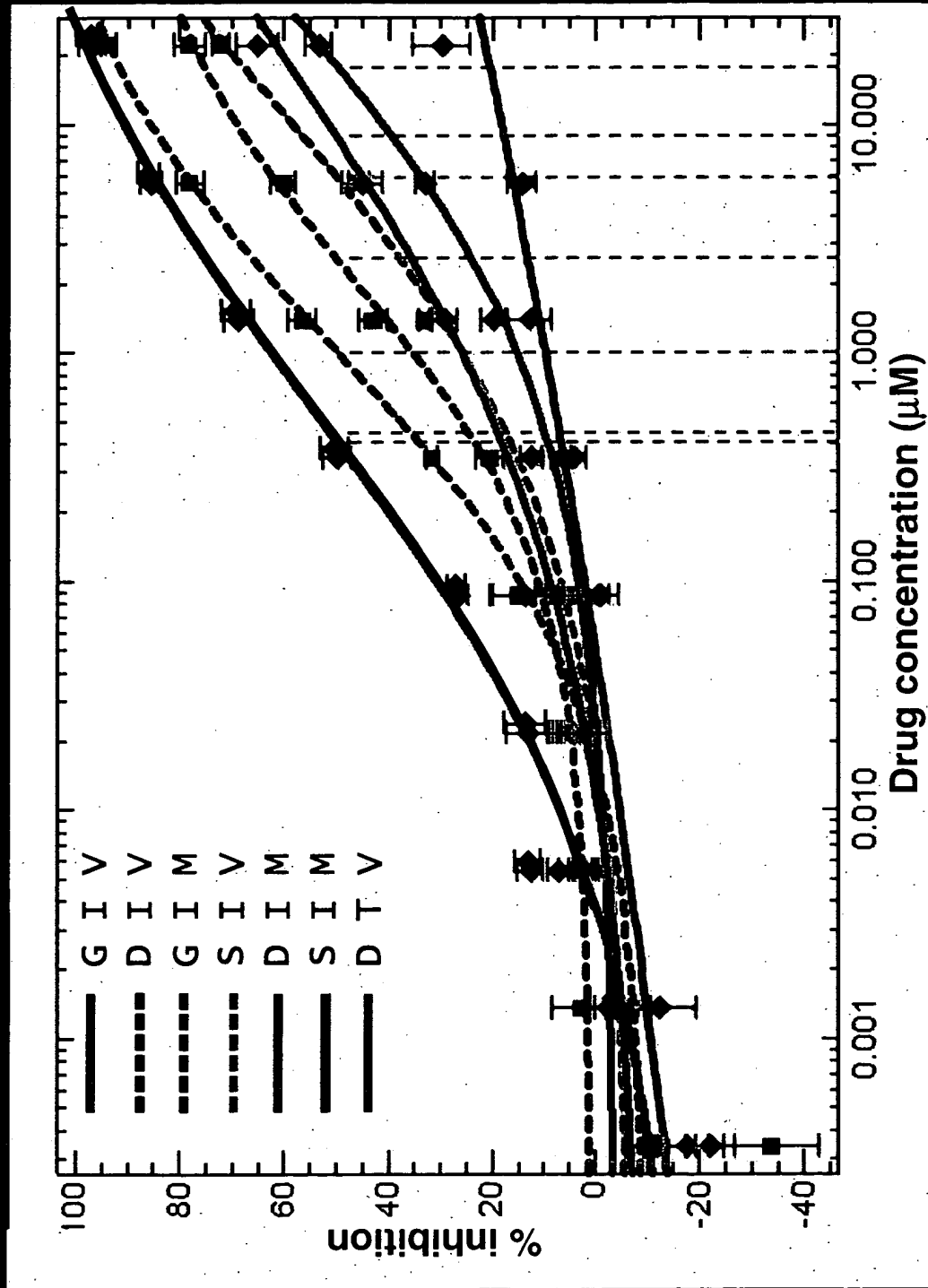
NL4-3: well-characterized X4 tropic strain

JRCSF: well-characterized R5 tropic strain

91US005.11: R5 tropic isolate obtained from the NIH AIDS Research and Reference Reagent Program (ARRRP)

92HT593.1: Dual tropic (X4R5) isolate obtained from the NIH ARRRP.

Reduced Susceptibility: Fusion Inhibitor



Reduced Susceptibility: Fusion Inhibitor

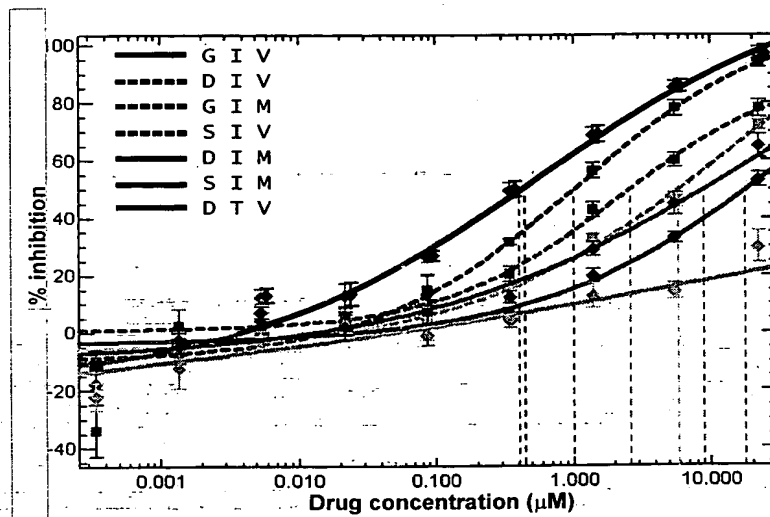


Figure 4B.

Measuring Entry Inhibitor Susceptibility: Drug Resistance Mutations

In this embodiment, reduced susceptibility to the fusion inhibitor T-20 conferred by specific drug resistance mutations in the gp41 envelope protein is demonstrated. Cells expressing CD4, CCR5 and CXCR4 were infected in the absence of T-20 and over a wide range of T-20 concentrations (x-axis log10 scale). The percent inhibition of viral replication (y-axis) was determined by comparing the amount of luciferase produced in infected cells in the presence of T-20 to the amount of luciferase produced in the absence of T-20. Isogenic viruses containing one or two specific mutations in the gp41 transmembrane envelope protein were tested (highlighted in red in the figure legend). Drug susceptibility is quantified by determining the concentration of T-20 required to inhibit 50% of viral replication (IC50, shown as vertical dashed lines). Viruses with lower IC50 values are more susceptible to T-20 than viruses with higher IC50 values.

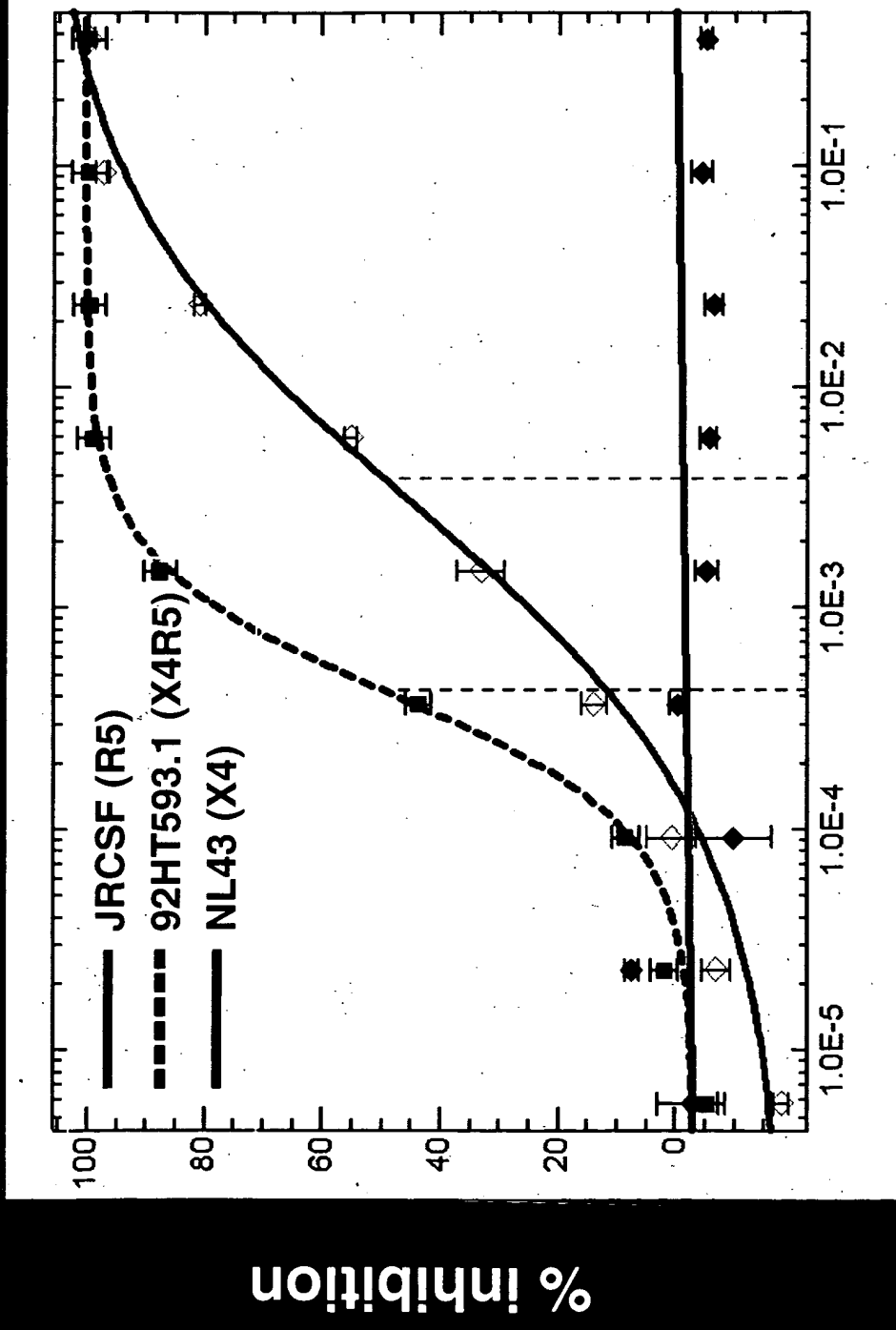
No mutation (wildtype sequence): GIV

Single mutations: GIV, DIM, SIV

Double mutations: DIM, SIM, DTV

Figure 5A

Entry Inhibitor Susceptibility: CCR5 Inhibitor



Drug: R5 Inhibitor
Cell: CD4/CCR5

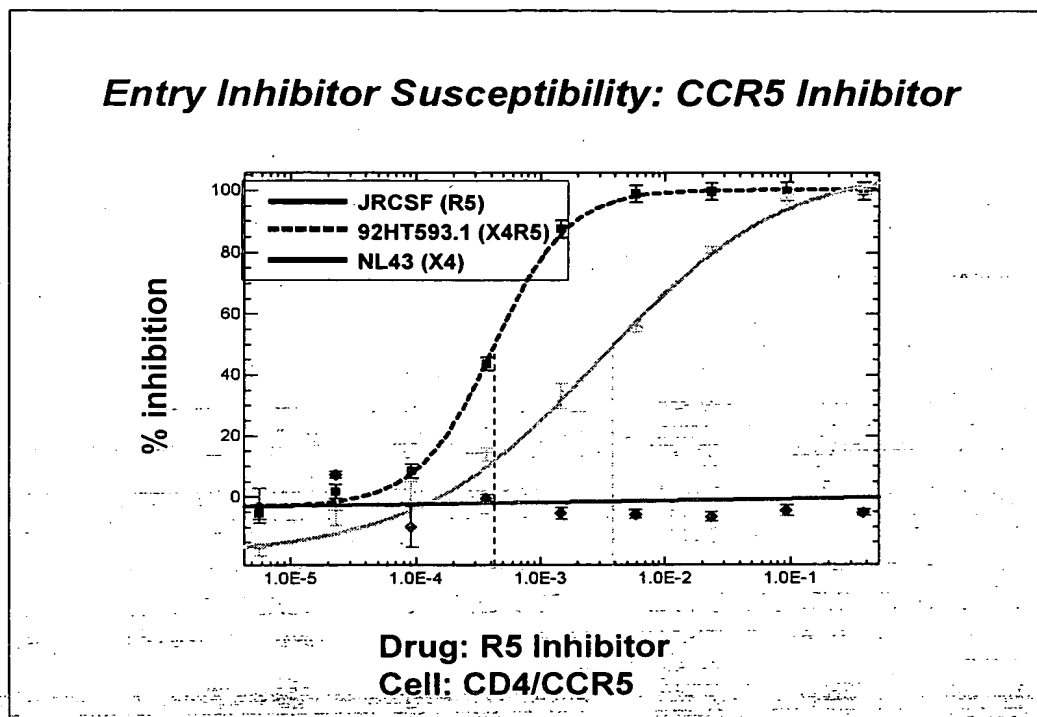


Figure 5A.

Measuring Entry Inhibitor Susceptibility: CCR5 Inhibitor

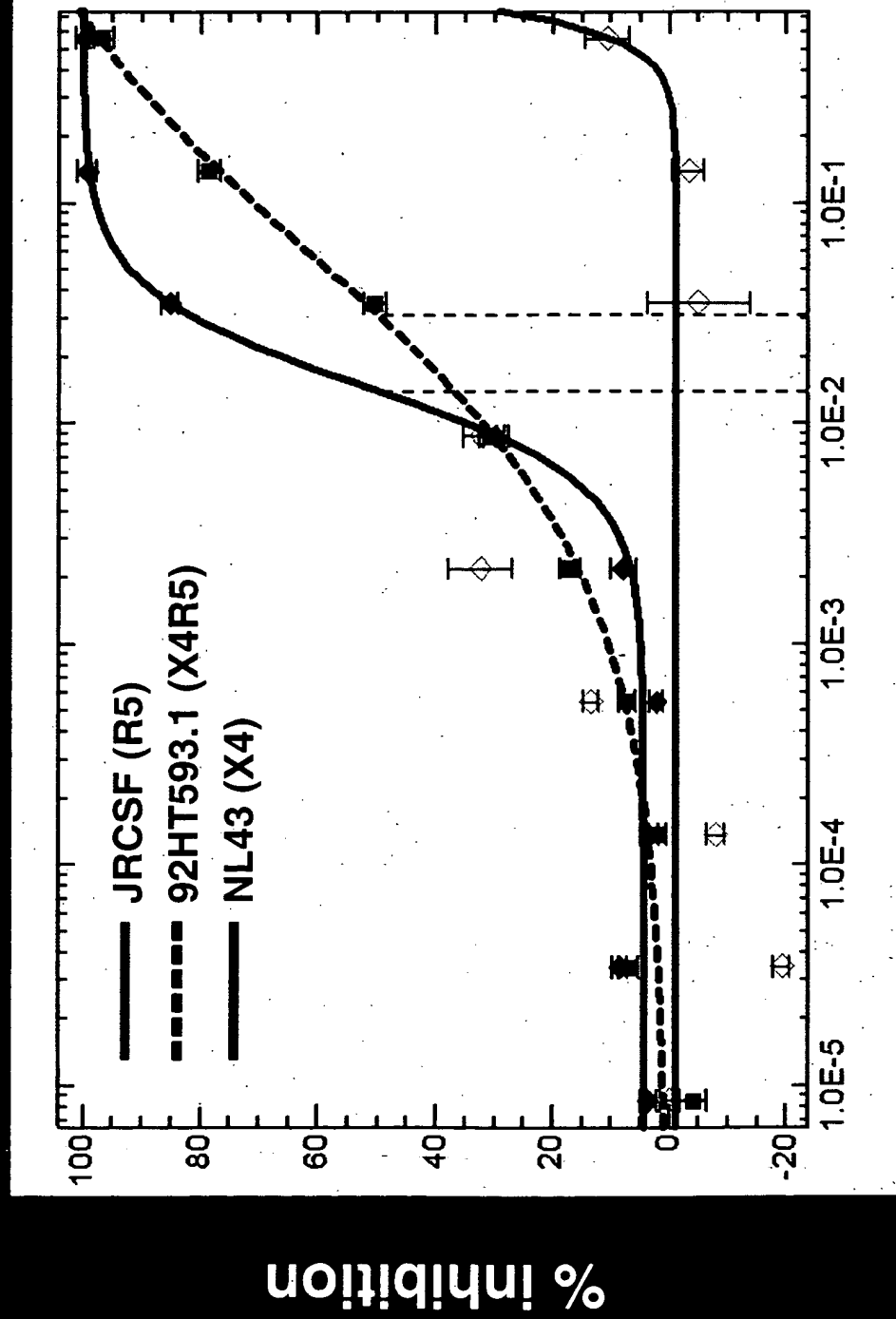
In this embodiment, susceptibility to a CCR5 inhibitor (merck compound) is demonstrated. Cells expressing CD4 and CCR5 (R5 cells) were infected in the absence of the CCR5 inhibitor and over a wide range of CCR5 inhibitor concentrations (x-axis log10 scale). The percent inhibition of viral replication (y-axis) was determined by comparing the amount of luciferase produced in infected cells in the presence of CCR5 inhibitor to the amount of luciferase produced in the absence of CCR5 inhibitor. R5 tropic, X4 tropic and dual tropic viruses were tested. Drug susceptibility is quantified by determining the concentration of CCR5 inhibitor required to inhibit 50% of viral replication (IC50, shown as vertical dashed lines). Viruses with lower IC50 values are more susceptible to the CCR5 inhibitor than viruses with higher IC50 values. The X4 tropic virus did not infect the R5 cells.

NL4-3: well-characterized X4 tropic strain

JRCsf: well-characterized R5 tropic strain

92HT593.1: Dual tropic (X4R5) isolate obtained from the NIH ARRRP.

Entry Inhibitor Susceptibility: CXCR4 Inhibitor



Drug: X4 Inhibitor
Cell: CD4/CXCR4

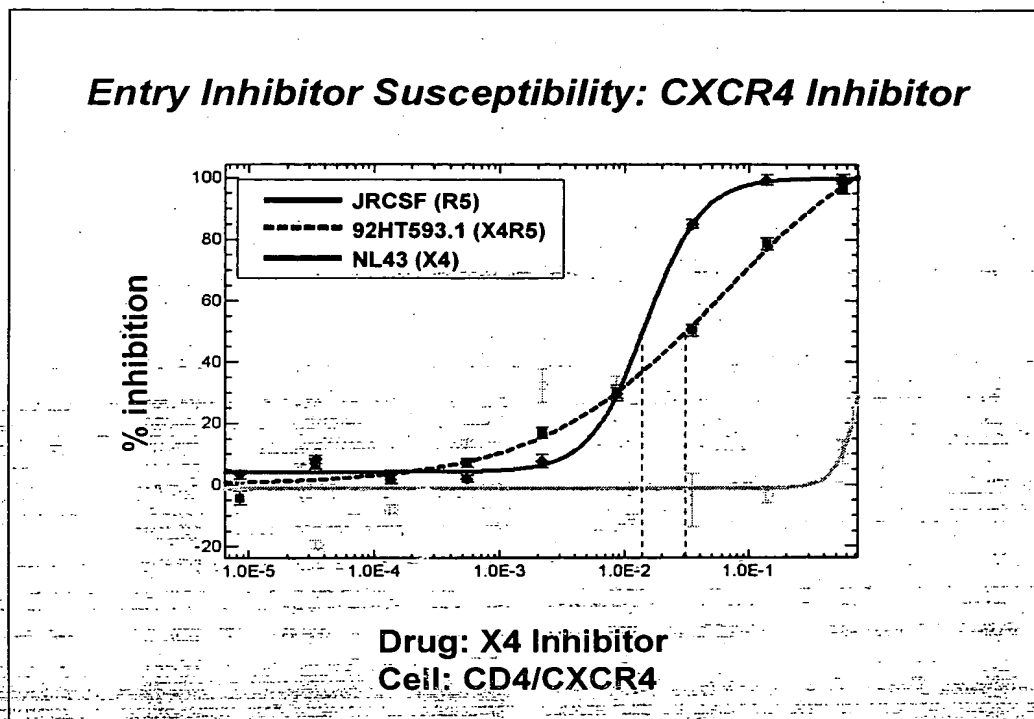


Figure 5B.

Measuring Entry Inhibitor Susceptibility: CXCR4 Inhibitor

In this embodiment, susceptibility to a CXCR4 inhibitor (AMD3100) is demonstrated. Cells expressing CD4 and CXCR4 (X4 cells) were infected in the absence of the CXCR4 inhibitor and over a wide range of CXCR4 inhibitor concentrations (x-axis log10 scale). The percent inhibition of viral replication (y-axis) was determined by comparing the amount of luciferase produced in infected cells in the presence of CXCR4 inhibitor to the amount of luciferase produced in the absence of CXCR4 inhibitor. R5 tropic, X4 tropic and dual tropic viruses were tested. Drug susceptibility is quantified by determining the concentration of CXCR4 inhibitor required to inhibit 50% of viral replication (IC₅₀, shown as vertical dashed lines). Viruses with lower IC₅₀ values are more susceptible to the CCR5 inhibitor than viruses with higher IC₅₀ values. The R5 tropic virus did not infect the X4 cells.

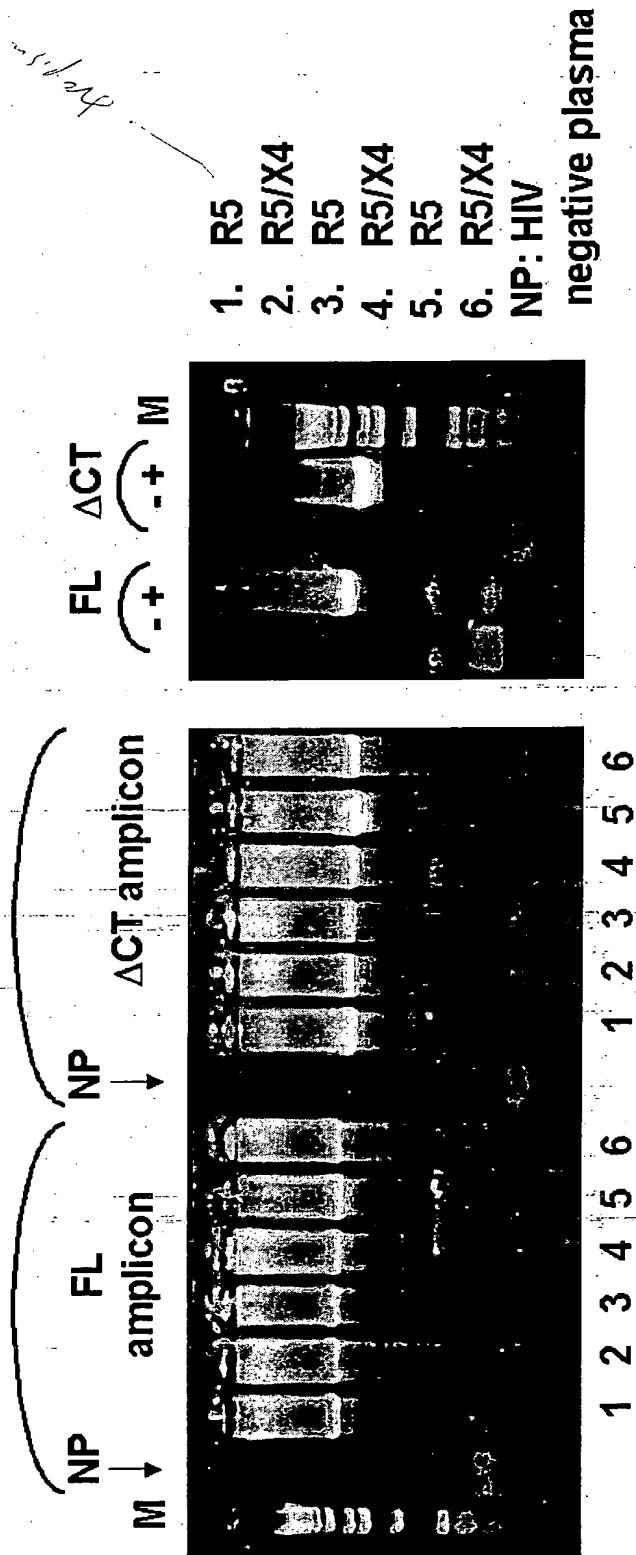
NL4-3: well-characterized X4 tropic strain

JRCSF: well-characterized R5 tropic strain

92HT593.1: Dual tropic (X4R5) isolate obtained from the NIH ARRRP.

Figure 6

2. Envelope Sequence Amplification



Co-Receptor Tropism	# of isolates
X4	15
R5	24
X4/R5	15
Undefined	35
Envelope Subtype	# of isolate
Clade A	2
Clade B	76
Clade C	7
Clade D	1
Clade E	3

Figure 7

3. Target Cell Receptor and Co-Receptor Expression

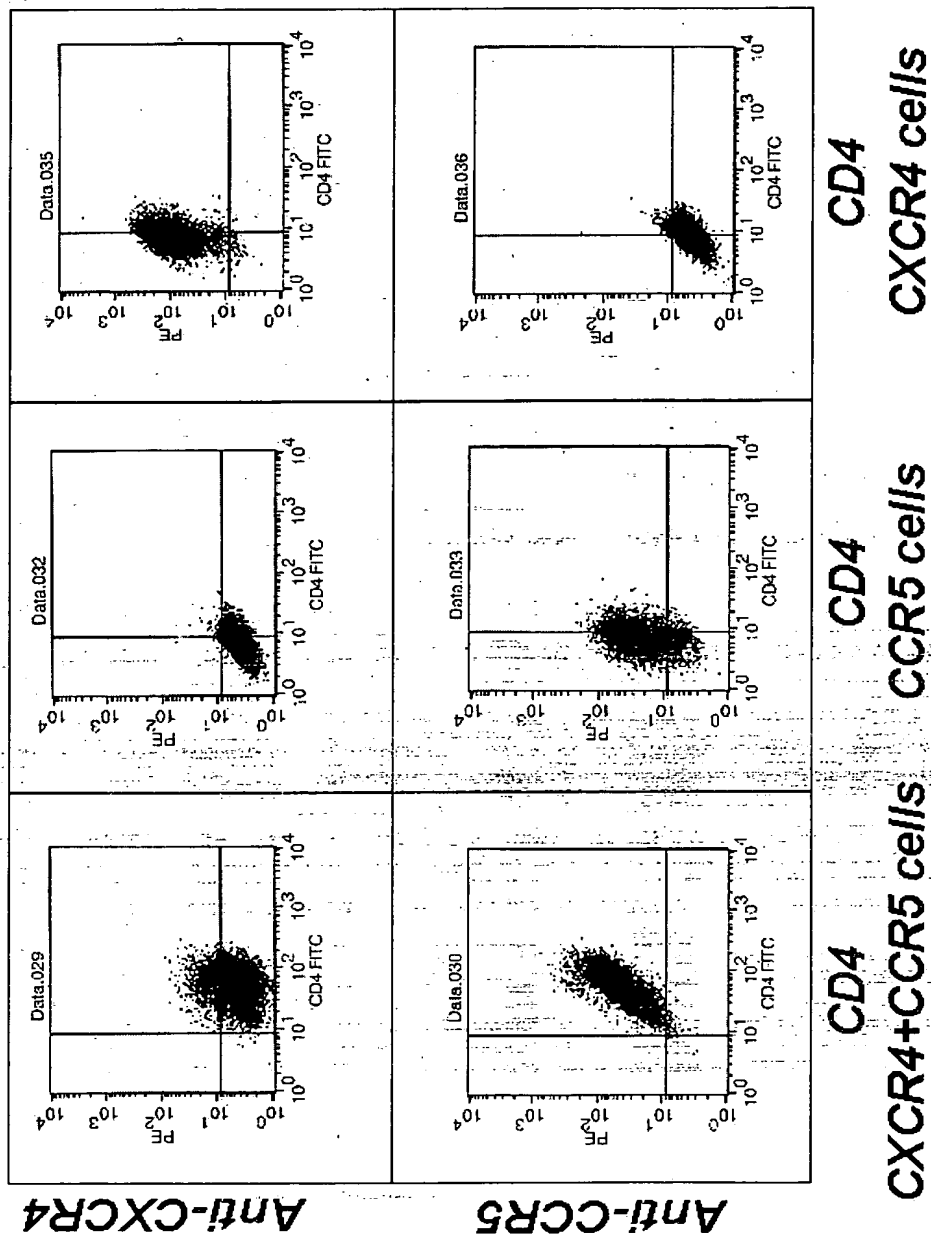
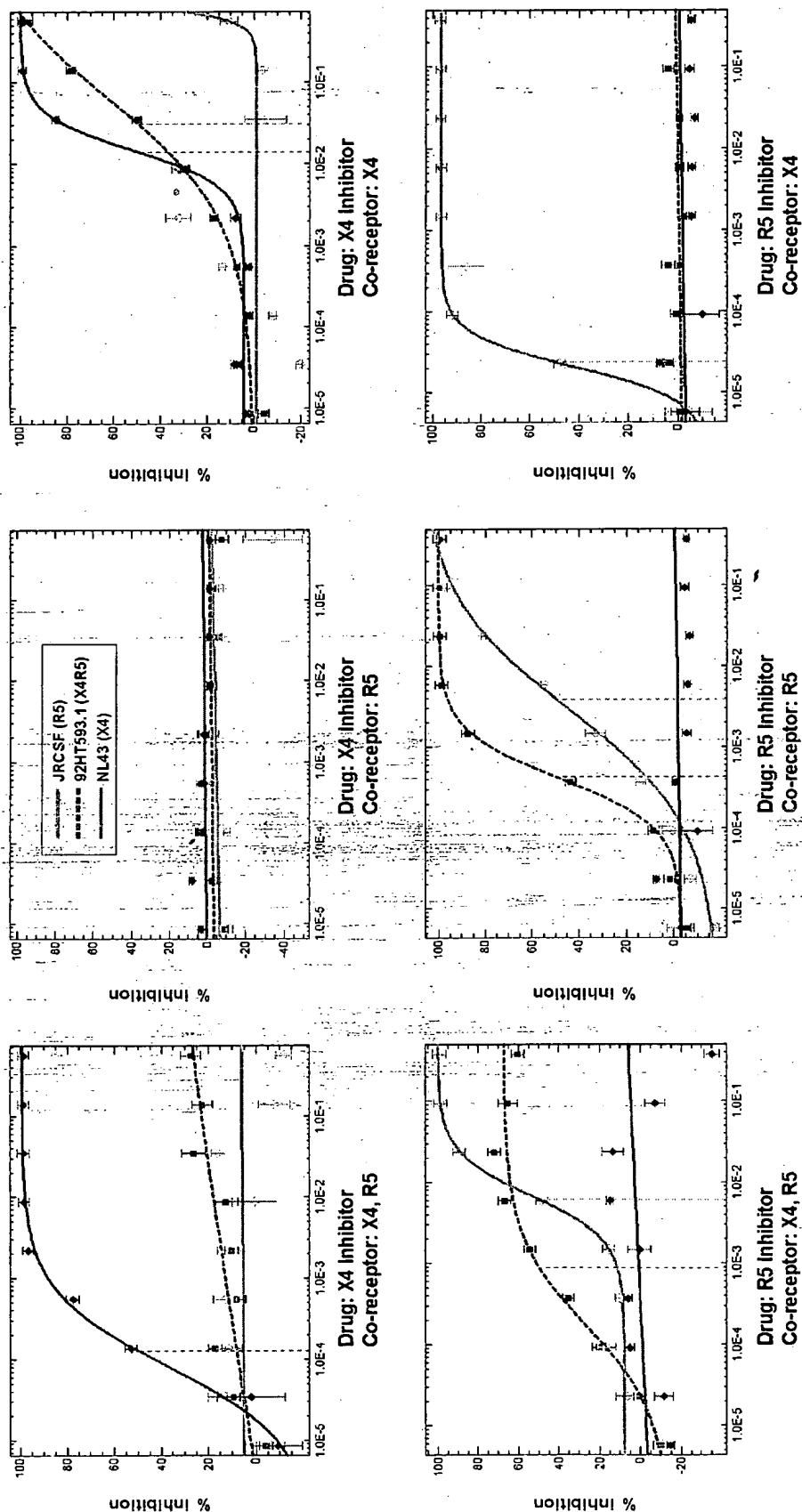


Figure 8

4.

Inhibition By Co-Receptor Antagonists



6. Inhibition By Membrane Fusion Inhibitor

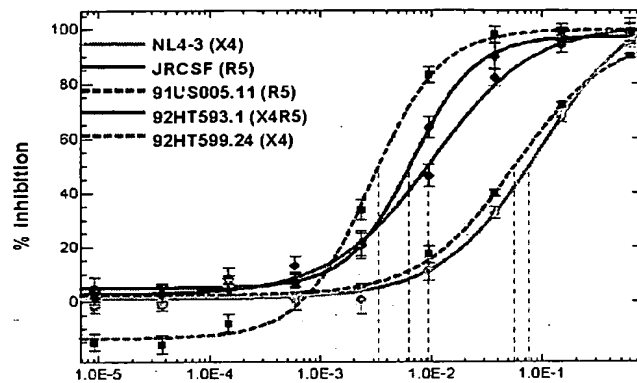


Figure 4A

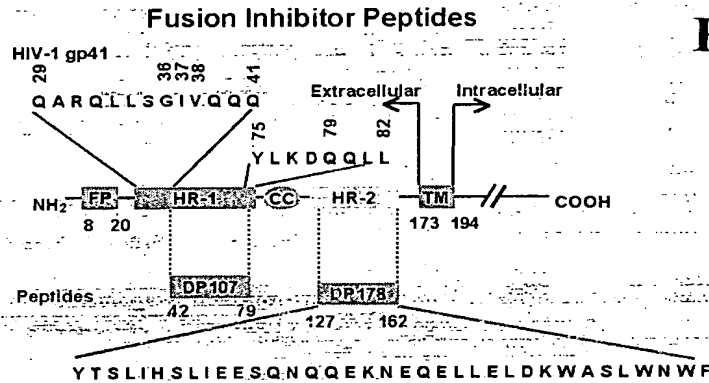


Figure 9

HIV-1 Site Directed Mutants

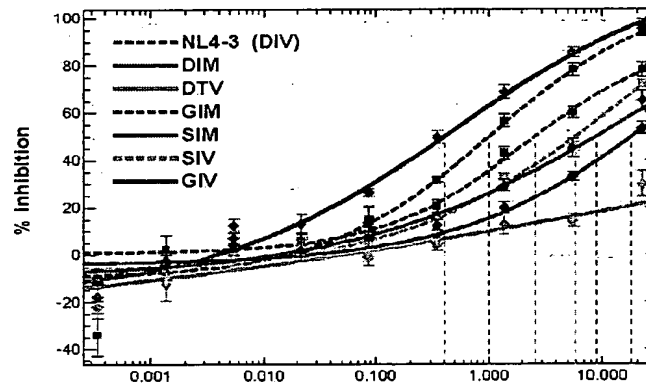


Figure 4B

SDM Virus	DP 178 Sens. ^a	Fold Change ^b
HXB2 G I V		1.0
NL4-3 G I V	S	5.2
NL4-3 D I V	S	12.8
NL4-3 S I V	S	74.2
NL4-3 G I M	S	33.0
NL4-3 D I M	R	113.0
NL4-3 S I M	R	227.4
NL4-3 D T V	R	>281.8
JRCSF G I V		2.1
JRCSF D I V		104.0

^a Rimsky et al., J. Virol. 72(2):986-993

^b Fold change in IC₅₀ (vs. HXB2) using PhenoSense HIV Entry Assay